Euphytica **104:** 119–125, 1998.

Genetic diversity among *Paspalum* spp. as determined by RFLPs

Robert L. Jarret[1], Zhaowei W. Liu[2] & Robert W. Webster[3]
[1] *USDA/ARS, Plant Genetic Resources, Georgia Experiment Station, 1109 Experiment Street, Griffin, GA 30223-1797 U.S.A.;* [2] *present address: Department of Crop and Environmental Sciences, Virginia Technological Institute and State University, Blacksburg, VA 24061 U.S.A.;* [3] *USDA/ARS/BA/NGRL, Building 003, BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705 U.S.A.*

Received 22 December 1997; accepted 25 June 1998

Key words: genetic diversity, *Paspalum*, restriction fragment length polymorphisms

Summary

The genus *Paspalum* is characterized by over 400 species that are indigenous to a wide range of stressful habitats and marginal environments. Fifty-one accessions representing 29 *Paspalum* species were analyzed for DNA restriction fragment length polymorphisms (RFLPs). Fifteen random genomic probes were used in combination with restriction enzyme *Eco*RI to detect RFLPs, and data were analyzed phenetically. Hybridization with the 15 selected clones resulted in the detection of 261 RFLPs. Among the 261 restriction fragments scored, 204 (78.2%) were phenetically informative. Extensive RFLP variation was found between the species studied. Species affinities based on RFLP data were found to be in close agreement with previously determined relationships based on both morphological and cytological characteristics.

Introduction

Paspalum L. is a member of the tribe Paniceae R. Br. Within the Paniceae, *Paspalum* is one of the largest and most complex genera, containing over 400 species that are largely endemic to the tropics and subtropics of the world (Chase, 1929; Clayton & Renvoize, 1986). The center of diversity of this genus is South America (Fernandes et al., 1968; Burkhart et al., 1969; Rosengurtt et al., 1970). The economic value of *Paspalum* is difficult to estimate, though it is assumed to be considerable. Several species (e.g. *P. fasciculatum* Flugge and *P. dilatatum* Poir.) are currently cultivated for grazing, hay production, and are often introduced species for pasture and range improvement. Species such as *P. vaginatum* and *P. distichum* provide for dune stabilization and waterfowl fodder under saline and fresh water environments, respectively. *Paspalum vaginatum* can be cultivated as a turfgrass or for soil stabilization. In Asia, *P. scrobiculatum* L. has been domesticated as a cereal grain.

Cytologically, most *Paspalum* species have a basic chromosome number of x = 10, with a few species being x = 6 or x = 9 (Mehra & Chaudhary, 1981; Pitman et al., 1987). Variation in DNA contents among *Paspalum* species is approximately 3-fold as determined by laser flow cytometry of DAPI-stained nuclei (Jarret et al., 1994). During the 1960s, cytological investigations of the *Paspalum* genome were undertaken in order to evaluate phylogenetic relationships. Phylogenetic relationships were determined by hybridizing selected parents and subsequently observing chromosome pairing during meiosis in the F_1 hybrids. Genomic relationships among a number of species have been studied in this manner and various genomes have been assigned designations (Burson, 1991b).

Members of the genus *Paspalum* share two striking similarities; the frequent occurrence of polyploidy and apomictic reproduction. Many *Paspalum* species exhibit polyploidy that varies among species both in frequency and range. Various ecotypes of *P. dilatatum* with 2n = 40, 50, or 60 have been reported (Burson, 1991b). Ecotypes of other species including *P. notatum, P. thunbergii* and *P. plicatulum* are common as 2n = 20 or 40 (Darlington & Wylie, 1961). The occurrence of polyploidy and apomixis hinders cyto-

logical studies of phylogeny, and also conventional breeding programs, due to the resultant difficulties associated with the production and identification of hybrid progeny.

The present study is an attempt to examine relationships among 29 *Paspalum* species using RFLPs (restriction fragment length polymorphisms). Relationships between species as determined by DNA analysis were compared with relationships determined from morphological and cytological studies. RFLP markers have been used for genetic diversity and phylogenetic studies in many crop taxa, including: *Arachis* L. (Kochert et al., 1991), *Brassica* L. (Song et al., 1988a, 1988b, 1990), *Ipomoea* L. (Jarret et al., 1992a), *Lycopersicon* Mill. (Miller & Tanksley, 1990), *Musa* L. (Gawel et al., 1992), Triticeae (Monte et al., 1993) and *Cajanus* (Nadimpalli et al., 1992).

Materials and methods

Plant material

The 51 accessions representing 29 species of *Paspalum* analyzed in this study are presented in Table 1 and were obtained from the S-9 Plant Germplasm Collection (Jarret et al., 1992b). Species were chosen based on the availability of germplasm and represent a cross-section of the morphological forms and variation found within the genus. Seeds were planted in plastic pots in a glasshouse, and grown to reproductive maturity. Voucher herbarium specimens were collected at flowering and identified by R.D. Webster (USDA/ARS, Beltsville).

RFLP assays

DNA for library construction and RFLP analysis was isolated as described by Liu & Furnier (1993). Approximately 5 μg of genomic DNA from each accession was digested to completion with 30 U of *Eco*RI for about 8 h at 37 °C. Restriction fragments were separated by electrophoresis on 1% agarose gels. DNA fragments were then transferred to MagnaGraph nylon membranes (Micro Separation Inc., Westborough, MA) using a Hybaid Vacu-Aid (National Labnet Co., Woodbridge, NJ) vacuum blotting apparatus, and the membranes were baked at 75 °C for 1.5 h. Procedures for probe labeling and hybridization were the same as those described by Liu & Furnier (1993).

A random genomic library was constructed from DNA of *P. elongatum* (PI # 404627). The methylation-sensitive restriction enzyme *Pst*I was used for library construction in order to enrich the library for single-copy sequences (Helentjaris et al., 1988). *Pst*I-digested genomic DNA was ligated into pUC19 and subsequently used to transform *E. coli* strain DH5α. To identify single-copy clone sequences, individual clones from the library were hybridized with ^{32}P-labeled total genomic DNA using a dot blot apparatus. Only those clones having a very weak or no hybridization signal were selected as probes for further use.

Data analysis

All restriction fragments detected by Southern analysis were treated as discrete characters. Autoradiograms were scored for the presence (1) or absence (0) of individual DNA fragments. Fragments that could not be scored unambiguously were scored by a missing data code. RFLP data were analyzed phenetically using NTSYS-pc version 1.70 (Rohlf, 1992). A Jaccard's (1908) similarity coefficient matrix was calculated and subjected to cluster analysis using unweighted pair-group method analysis (UPGMA). A cophenetic value matrix was computed using the similarity matrix, and was compared with the matrix using the Mantel matrix correspondence test (Sneath & Sokal, 1973).

Results and discussion

Banding patterns

Thirty *Paspalum* genomic clones were screened in order to identify those capable of detecting polymorphisms when hybridized to DNA from a range of *Paspalum* accessions digested with *Eco*RI. Fifteen clones were selected for further use. The remainder of the clones generated either faint hybridization signals or complex banding patterns that could not be scored with confidence. Hybridization with the 15 selected clones resulted in the detection of 261 RFLPs among the 51 accessions. Among the restriction fragments observed, three (1.1%) were common to all accessions, 54 (20.7%) were unique to a single accession, and 204 (78.2%) were shared by two or more accessions and were phenetically informative (Figure 1).

Table 1. Plant materials used in the study

Paspalum species	PI No.	Abbr.	Origin
P. commune Lillo	508646	commun	Tucuman, Argentina
P. conjugatum Berg.	508663	conjug	Missiones, Argentina
P. conspersum Schrad.	404646	conspe1	Puerto Presidente Strossner, Paraguay
	508658	conspe2	Missiones, Argentina
P. cromyorrhizon Doell	508666	cromyo	Corrientes, Argentina
P. densum Poir.	284510	densum	Puerto Rico
P. dilatatum Poir.	462265	dilata	Uraguay
P. distichum L.	508728	distic1	Buenos Aires, Argentina
	508768	distic2	Corrientes, Argentina
	284500	distic3	Chile
P. elongatum Griseb.	404627	elonga	Chaco-I, Paraguay
P. exaltatum Presl	404683	exalta1	Caapucu, Paraguay
	404848	exalta2	Minas, Uruguay
P. guaraniticum Parodi	404852	guaran	Pay sandu, Uruguay
P. guenoarum Arech.	303986	guenoa1	Pelotas, Brazil
	404883	guenoa2	Pay sandu, Uruguay
	303988	guenoa3	Brazil
P. humboldtianum Fluegg	508762	humbol	Cordoba, Argentina
P. lividum Trin.	404638	lividu1	Chaco, Paraguay
	404637	lividu2	Rio Paraguary, Paraguay
	508937	lividu3	Corrientes, Argentina
	508668	lividu4	Formosa, Argentina
P. macrophyllum Kunth	282807	macrop	Paraguay
P. malacophyllum Trin.	337586	malaco1	Corrientes, Argentina
	315731	malaco2	Florida, U.S.A.
	508956	malaco3	Molina Punta, Argentina
P. mandiocanum Trin.	508809	mandio1	Rio Grande do Sul, Brazil
	508816	mandio2	Santa Catarina, Brazil
P. nicorae Parodi	310131	nicora1	Uruguaiana, Brazil
	283020	nicora2	Tranqueras, Uruguay
P. notatum Fluegge	462276	notatu1	Rocky Virgin Field, Uruguay
	424652	notatu2	Corrientes, Argentina
	508849	notatu3	Isla el Timbo, Argentina
P. paniculatum L.	462279	panicu1	Tranqueras, Uruguay
	304001	panicu2	Brazil
	310192	panicu3	Nova Fac. Brazil
P. plicatulum Michx.	162905	plicat	Asuncion, Paraguay
P. pubiflorum Fourn.	304147	pubifl	Lagos-Aquascalientes, Mexico
P. quadrifarium Lamk.	404364	quadri1	Vacaria, Brazil
	508947	quadri2	Buenos Aires, Argentina
	310049	quadri3	Nova Fac. Brazil
P. scrobiculatum L.	464146	scrobi	India
P. tenellum Willd.	308572	tenell	Merida, Venezuela
P. thunbergii Steud.	435995	thunbe	Japan
P. unispicatum (Scribn. & Merr.) Nash	509007	unispi	Salta, Argentina
P. urvillei Steud.	508664	urvill1	Parana, Brazil
	462304	urvill2	Cerro Largo, Uruguay
	509014	urvill3	Cordoba, Brazil
	415166	urvill4	Corrientes, Argentina
P. vaginatum Swartz	509018	vaginm	Ao del Pueblo, Argentina
P. yaguaronense Henr.	310270	yaguar	Pelotas, Brazil

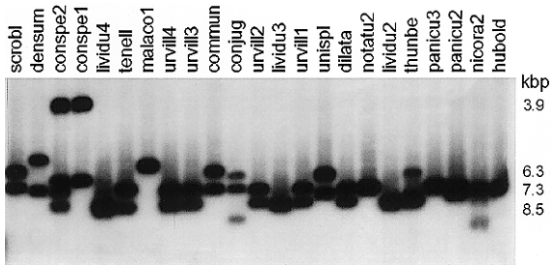

Figure 1. Autoradiograph depicting hybridization of clone P-66 to DNA from a subset of *Paspalum* species digested with *Eco*RI. Names are abbreviated as per Table 1.

Variation between Paspalum *species*

Twelve of the 29 *Paspalum* species in the study were represented by more than a single accession (Table 1). RFLP variation was readily detected among accessions within most of these 12 species. Accessions within *P. exaltatum, P. guenoarum, P. lividum, P. malacophyllum, P. notatum,* and *P. quadrifarium* shared less than 60% of their DNA fragments, yet accessions within species tended to cluster together on the phenogram (Figure 2). These results are not totally unexpected since accessions were collected over large geographic regions where they may have been subject to a variety of selection pressures. Jaccard's similarity coefficients between species ranged from 0.15 to 0.45, indicating a high level of genetic variation among the 29 species analyzed. The correlation coefficient (*r*) of the cophenetic value matrix with its cluster matrix was 0.91, indicating a very good fit of the matrices (Rohlf, 1992).

Phenetic analysis and morphological taxonomy

To facilitate a discussion of the relationships among taxa, the phenogram derived from RFLP data (Figure 2) was truncated at the 20% similarity level. The species were then divided into six clusters and are discussed below. Results from the phenetic analysis of the RFLP data set illustrate a strong correlation with morphological similarities among the species.

Cluster 1. The cluster consists of 10 species. The phenogram divides this cluster into two subclusters, one containing *P. pubiflorum, P. urvillei, P. dilatatum*, and *P. thunbergii*, and the other containing *P. mandiocanum, P. paniculatum, P. tenellum, P. conspersum, P. macrophyllum*, and *P. commune. Paspalum dilatatum* and *P. urvillei* have been widely introduced in the tropics and warm temperate regions of the world, but are native to the southeastern United States and South America. These species are characterized by their numerous primary branches and paired spikelets with a hair-fringed second glume (Chase, 1929). *Paspalum thunbergii*, a native to tropical Asia is characterized by numerous racemes and paired spikelets. Morphologically, *P. thunbergii* apparently is properly grouped with *P. dilatatum. Paspalum pubiflorum* differs from *P. lividum* in possessing a distinctly convex second glume and upper lemma, solitary and hairy spikelets, and a coarser textured upper floret. Analysis of the RFLP data indicate that *P. pubiflorum* has a closer affinity to *P. dilatatum* and relatives than to *P. lividum*. Cytogenetic studies also support this result, as discussed below.

The other subcluster of Cluster 1 consists of six species. Morphologically, *P. tenellum* and *P. paniculatum* are very similar and are distinguished based on variation in spikelet length. *Paspalum mandiocanum* is similar to *P. paniculatum* and is also differentiated based on spikelet length (Chase, 1929). *Paspalum macrophyllum* and *P. commune* were not treated by Chase (1929) and little is known about these latter two species. However, *P. macrophyllum* is likely best treated as a synonym of *P. conspersum*.

Cluster 2. This cluster includes five species (*P. elongatum, P. malacophyllum, P. humboldtianum, P. plicatulum*, and *P. densum*). The most distinctive member of this cluster is *P. humboldtianum* that was previously placed in subgenus *Ceresia* and which was described on the basis of a broadly winged primary branch, pale upper floret, and spikelets fringed with long silky hairs (Chase, 1929). *Paspalum humboldtianum* is not a typical member of this group, since it lacks the broadly winged primary branch. The most similar members of this cluster are *P. elongatum* and *P. malacophyllum*, which have an elongate main axis with numerous primary branches, reduced glumes, and ridged upper lemmas. *Paspalum densum* and *P. plicatulum* are relatively dissimilar morphologically (Chase, 1929).

Cluster 3. This cluster consists of four species (*P. quadrifarium, P. exaltatum, P. guaraniticum*, and *P. cromyorrhizon*) which are, at present, not systematically grouped within the genus. These taxa, together with others not included in this study, form a complex of morphologically and closely related South American species. *Paspalum cromyorrhizon* is similar to *P. guaraniticum* and primarily differentiated from it on the tridentate second glume.

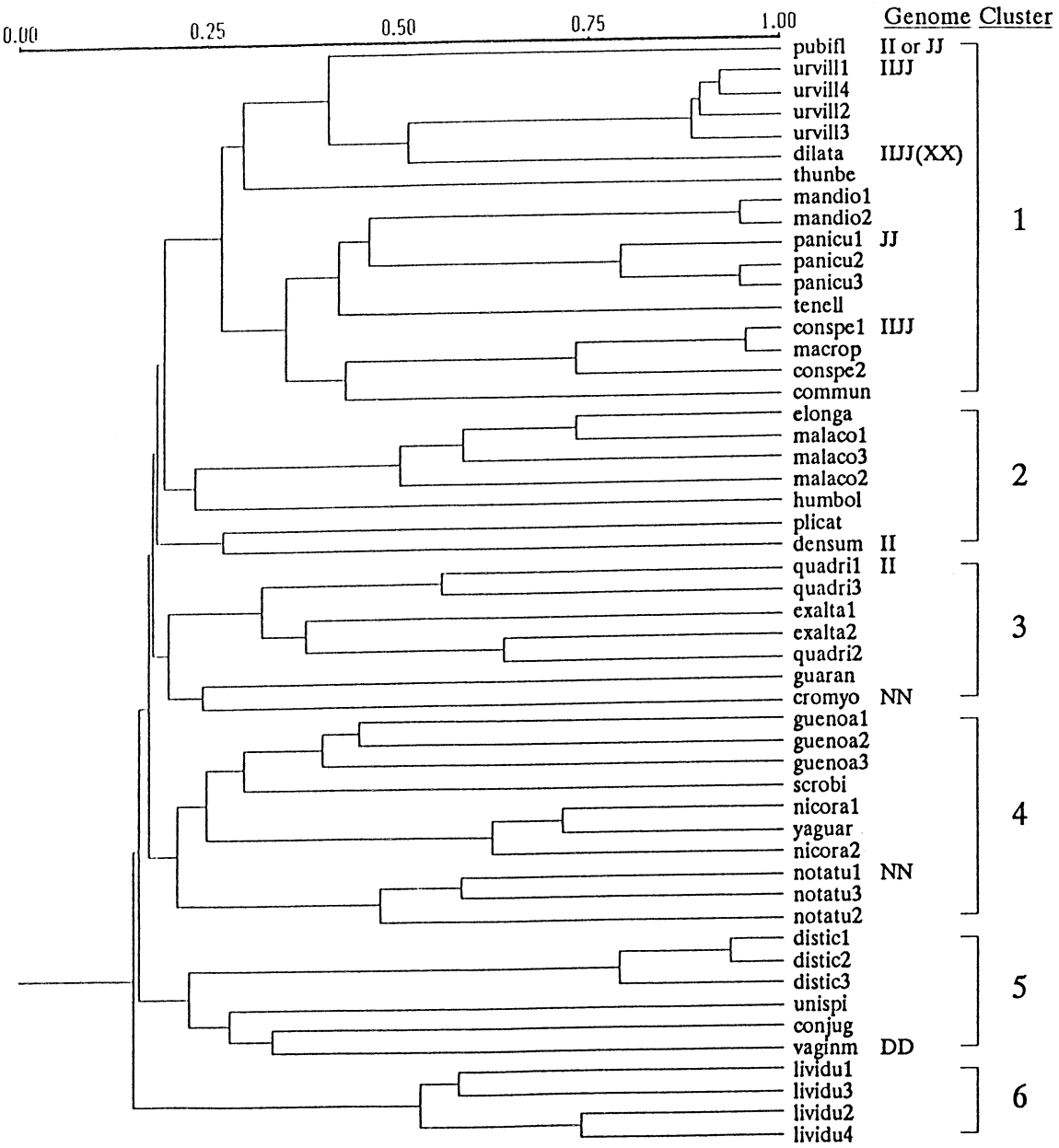

Figure 2. RFLP-derived phenogram depicting relationships among *Paspalum* species. Accessions within species are indicated by numbers following the species. Genome classifications are from: Burson (1978, 1979, 1981, 1991a, 1991b, 1992), Caponio & Quarin (1990, 1993), Quarin & Norrmann (1990) and Quarin et al. (1984).

Cluster 4. This cluster consists of five species (*P. guenoarum, P. scrobiculatum, P. nicorae, P. notatum*, and *P. yaguaronense*). *Paspalum notatum* was placed in group Notata, which was defined by Chase (1929) as perennials with two conjugate primary branches and solitary spikelets. The other species of this group do not possess conjugate racemes; however, they do share common features with each other and *P. notatum*. Common characteristics of these closely related species include the presence of a pronounced main axis, solitary spikelets, a dark brown smooth upper floret, and frequently with minute transverse ridges on the face of the lemma of the lower floret.

Cluster 5. This cluster contains four species, *P. distichum, P. unispicatum, P. conjugatum,* and *P. vaginatum*. A distinction between *P. distichum* and *P. vaginatum* is their fresh water native habitat (*P. distichum*, Van der Valk, 1993) or salt or saline ecosystems (*P. vaginatum*, Lakanmie & Okusanya, 1990), respectively. *Paspalum conjugatum* is characterized by lacking a main axis and two yellow primary branches. *Paspalum unispicatum* is characterized by an inflorescence reduced to a single primary branch and first glume present. However, *P. unispicatum* is often described as variable for the latter character. The unifying characteristic that differentiates these four species from other taxa is the complete absence of the main axis. Results from the present RFLP study indicate that these species, in which the inflorescence branches originate from a single point or in which the inflorescence is reduced to a single racemose primary branch, are closely related and are best treated as belonging to the same generic subgroup.

Cluster 6. The last cluster consists of four accessions of *P. lividum*, a species occurring in the southeast United States, Mesoamerica, and south to Argentina. Chase (1929) placed this species in group Livida, a poorly defined group consisting of eight species with perennial habit, compressed culms, flat leaf blades and spikelets 2 to 3.1 mm long. Additional diagnostic features of *P. lividum* include the glabrous, paired, elliptic to obovate spikelets, and non-conjugate primary branches.

Phenetic analysis and cytological identity

Cytological studies of a number of *Paspalum* species have been made in order to investigate their cytotaxonomic relationships. Relationships were determined based on the extent of chromosome pairing during meiosis in interspecific hybrids. Letters were assigned to the various genomes based on chromosome pairing characteristics. To date, four different genomes, I, J, D, and N, have been identified in the genus (Burson, 1992). The genome relationships of 10 of the 29 species in this study have been previously investigated and their genome identities are presented in Figure 2. Results from the RFLP analysis show interesting similarities with relationships determined cytologically.

Cluster 1 (Figure 2) contains five species (*P. conspersum, P. dilatatum, P. paniculatum, P. pubiflorum*, and *P. urvillei*) that have been studied cytologically and that possess either the I, J, or I and J genomes. *Paspalum densum* (Cluster 2) and *P. quadrifarium* (Cluster 3) are designated as I genome. The N and D genomes, which are distinctly different from the I or J genomes, are distributed in Clusters 3, 4, and 5. These genome classifications, based on cytological observations, correlate closely with our RFLP-derived phenogram (Figure 2).

Based on the genome relationships observed, Burson (1991b) considered *P. urvillei* as one of possible progenitors of *P. dilatatum*. The results of our RFLP analysis indicate a close relationship between these two species (Figure 2). Since species with the same genome classification normally cluster together, we speculate that *P. thunbergii* and *P. macrophyllum*, which have close affinity to *P. dilatatum* (IIJJXX) and its relatives, and to *P. conspersum* (IIJJ) based on the phenogram (Figure 2), respectively, possess genomes with homology or partial homology to I and/or J genomes.

In summary, phenetic analysis of RFLP data was used to determine relationships among 29 *Paspalum* species. Placement of individual taxa on the RFLP-derived phenogram are in general agreement with previous studies based on analysis of morphological and cytological characteristics. Data presented indicate that RFLPs are useful in efforts to examine relationships among *Paspalum* species, and that similar studies across a broader range of taxa would provide much needed information on genetic relationships within this genus.

Acknowledgements

We wish to acknowledge R.R. Duncan for access to plant material and editorial comments. Publication credits associated with this manuscript are shared equally by all authors.

References

Burkhart, A., J.A. Caro, K.A. Okada, R.A. Palacios & Z.E.R. Agrasar, 1969. Flora Illustrada de Entre Rios (Argentina): Part II, Gramineas. I.N.T.A. 4, Buenos Aires.

Burson, B.L., 1978. Genome relations between *Paspalum conspersum* and two diploid *Paspalum* species. Can J Genet Cytol 20: 365–372.

Burson, B.L., 1979. Cytogenetics of *Paspalum urvillei* × *P. intermedium*, and *P. dilatatum* × *P. paniculatum* hybrids. Crop Sci 19: 534–538.

Burson, B.L., 1981. Cytogenetic relations between *Paspalum jurgensii* and *P. intermedium, P. vaginatum*, and *P. setaceum* var. *ciliatifolium*. Crop Sci 21: 515–519.

Burson, B.L., 1991a. Homology of chromosomes of the X genomes in common and Uruguayan dallisgrass, *Paspalum dilatatum*. Genome 34: 950–953.

Burson, B.L., 1991b. Genome relationships between tetraploid and hexaploid biotypes of dallisgrass, *Paspalum dilatatum*. Bot Gaz 152 (2): 219–223.

Burson, B.L., 1992. Cytogenetic relationships between *Paspalum dilatatum* and *P. cromyorrhizon, P. indecorum*, and *P. laxum*. Int J Plant Sci 153 (2): 244–249.

Caponio, I. & C.L. Quarin, 1990. Intra- and interspecific hybridization between dallisgrass and vaseygrass. Crop Sci 30: 362–364.

Caponio, I. & C.L. Quarin, 1993. Cytology and reproduction of *Paspalum densum* and its genomic relationship with *P. intermedium* and *P. urvillei*. J Hered 84 (3): 220–222.

Chase, A., 1929. The North American species of *Paspalum*. Contr US Nat Herb 28: 1–310.

Clayton, W.D. & S.A. Renvoize, 1986. Genera Graminum: Grasses of the World. Royal Bot Gard, Kew, London. Kew Bull Add Ser XIII.

Darlington, C.D. & A.P. Wylie, 1961. Chromosome Atlas of Flowering Plants. Hafner Pub Co, New York.

Fernandes De Mores M.I.B.I.L., L. Barreto & F.M. Salzano, 1968. Cytological and evolutionary relationships in Brazilian forms of *Paspalum* (Gramineae). Caryologia 27: 454–465.

Gawel, N.J., R.L. Jarret & A.P. Whittemore, 1992. Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of *Musa*. Theor Appl Genet 84: 286–290.

Helentjaris, T., D. Weber & S. Wright, 1988. Identification of the genomic locations of restriction fragment length polymorphisms. Genetics 118: 353–363.

Hitchcock , A.S., 1935. Manual of the Grasses of the United States. US Dept Agric Misc Publ 200, Washington, DC.

Hitchcock, A.S., 1951. Manual of the Grasses of the United States. 2nd ed. Revised by A. Chase. US Dept Agric Misc Publ 200, Washington, DC.

Jaccard, P., 1908. Etude comparative de la distribution florale dans une portion des Alpes et des Jura. Bulletin de la Société Vaudoise des Sciences Naturelles 37: 547–579.

Jarret, R.L., N.J. Gawel & A.P. Whittemore, 1992a. Phylogenetic relationships of the sweetpotato (*Ipomoea batatas* (L.) Lam.). J Amer Soc Hort Sci 117 (4): 633–637.

Jarret, R.L., M. Spinks, G. Lovell & A.G. Gillaspie, 1992b. The S-9 plant germplasm collection. Diversity 6: 23–25.

Jarret, R.L., P. Ozias-Akins, S, Phatak, R. Nadimpalli, J.R. Duncan & S. Hilliard, 1994. DNA contents in *Paspalum* species determined by flow cytometry. Genet Res Crop Evol 4: 12–19.

Kochert, G., T. Halward, W.D. Branch & C.E. Simpson, 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor Appl Genet 81: 565–570.

Lakanmi, O.O. & O.T. Okusanya, 1990. Comparative ecological studies of *Paspalum vaginatum* and *Paspalum orbiculare* in Nigeria. J Trop Ecol 6: 103–114.

Liu, Z. & G.R. Furnier, 1993. Inheritance and linkage of allozymes and restriction fragment length polymorphisms in trembling aspen. J Hered 84 (5): 419–424.

Mehra, P.N. & J.D. Chaudhary, 1981. Male meiosis in some grasses of the tribe Paniceae from northeastern India. I. Genus *Paspalum*. Cytologia 46: 265–278.

Miller, J.C. & S.D. Tanksley, 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor Appl Genet 80: 437–448.

Monte, J.V., C.L. McIntype & J.P. Gustafson, 1993. Analysis of phylogenetic relationships in the Triciceae tribe using RFLPs. Theor Appl Genet 86: 649–655.

Nadimpalli, R.G., R.L. Jarret, S. Phatak & G. Kochert, 1992. Phylogenetic relationships of the pigeonpea (*Cajanus cajan*) based on nuclear restriction fragment length polymorphisms. Genome 36: 216–223.

Pilger, S., 1940. Gramineae. Nat Pfl Fam, ed 2, vol 14e: 58–67.

Pitman, M.W., B.L. Burson & E.C. Bashaw, 1987. Phylogenetic relationships among *Paspalum* species with different base chromosome numbers. Bot Gaz 148 (1): 130–135.

Quarin, C.L., B.L. Burson & G.W. Burton, 1984. Cytology of intra- and interspecific hybrids between two cytotypes of *Paspalum notatum* and *P. cromyorrhizon*. Bot Gaz 145: 420–426.

Quarin, C.L. & G.A. Norrmann, 1990. Interspecific hybrids between five *Paspalum* species. Bot Gaz 145: 366–369.

Rohlf, F.J., 1992. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Exeter Software, New Yok.

Rosengurtt, B., B. Arrillaga de Maffei & P. Izaguirre de Artucio, 1970. Gramineas Uruguayas. Universidad de la Republica, Dept. de Publicaciones, Montevideo, Uruguay.

Sneath, P.H.A. & R.R. Sokal, 1973. Numerical Taxonomy. W.H. Freeman and Company, San Francisco.

Song, K.M., T.C. Osborn & P.H. Williams, 1988a. *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 1. Genome evolution of diploid and amphidiploid species. Theor Appl Genet 75: 784–794.

Song, K.M., T.C. Osborn & P.H. Williams, 1988b. *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 2. Preliminary analysis of subspecies within *B. rapa* (syn. *campestris*) and *B. oleracea*. Theor Appl Genet 76: 593–600.

Song, K.M., T.C. Osborn & P.H. Williams, 1990. *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 3. Genome relationships in *Brassica* and related genera and the origin of *B. oleracea* and *B. rapa* (syn. *campestris*). Theor Appl Genet 79: 497–506.

Van der Valk, A.G., B.A. Middleton, R.L. Williams, D.H. Mason & C.B. Davis, 1993. The biomass of an Indian monsoonal wetland before and after being overgrown with *Paspalum distichum* L. Vegetatio 109: 81–90.